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(54) Title: A PROCESS FOR PRODUCING BETA-CASEIN ENRICHED PRODUCTS

(57) Abstract

The invention relates to a process of extracting a β-casein enriched product and a β-casein depleted product from a casein solids feedstock. The casein feedstock is slurried and cooled to a temperature range of -10 °C to 14 °C until the desired amount of β-casein has been dissolved and it is then separated from solid β-casein depleted product. For rennet casein feedstock the pH of the slurry is maintained at pH 5.0 - 8.0 while for other casein feedstock a pH of 3.5 to 8.0 may be used. The β-casein enriched product has a number of non-food and food uses including as an additive to infant formulations. The β-casein depleted product can be used for most of the same purposes as the casein feedstock.

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A PROCESS FOR PRODUCING BETA-CASEIN ENRICHED PRODUCTS

TECHNICAL FIELD

This invention relates to a method of producing a β-casein enriched product and a β-casein depleted product from casein curd or powder and to products produced by the method.

BACKGROUND ART

Casein may be obtained from mammalian milk by known techniques. For example, acid casein is obtained by acidifying milk with mineral or organic acids. Rennet casein is obtained by precipitation of the casein micelles of mammalian milk after hydrolysis of κ-casein with calf rennet or equivalent enzymes of bacterial, fungal or plant origin. Methods of commercial production of caseins are described in references 1 to 4 and 6.

One of the known fractions of casein is β-casein. β-casein has many properties which make it a commercially desirable product to obtain. Known properties include:

1. an ability to bind water;
2. a strong hydrophobic nature giving rise to foaming and emulsifying properties;
3. nutritional properties such as being a source of lysine and tryptophan;
4. pharmaceutical properties such as an ability to generate phosphopeptides affecting the level of mineral absorption in the intestine and an ability to form β-casomorphine which exhibits beneficial physiological activity;
5. adhesive, binding, rheological and viscosity lowering properties resulting from its molecular structure; and
6. dissimilar chemical and physical properties from those of other casein fractions and whole casein.

Human and cow milk are known to differ significantly in casein composition. In human milk the casein fraction consists almost entirely of β-casein. With bovine milk β-casein is present in smaller amounts and the major component is α_s-casein. β-casein enriched products have therefore been much sought after because they are the preferred casein ingredient for use in infant formula.

β-casein is a phosphorylated protein which, upon digestion in the human gut, can release peptides which exert specific physiological effects. These include peptides which have opioid activity. These properties allow β-casein to be used in both non-food and food products such as in low allergenic foods, low lactose dairy drinks, and pharmaceutical products.

Isolation of individual casein proteins (that is. the casein fractions) from mammalian milk is a well known art. A number of laboratory based methods have been published in which one or several of five different technologies have been used: differential solubility, liquid/liquid extraction, electrophoresis, membrane techniques, and chromatographic separations. Details of such technologies may be found in reference 7.

Japanese patent specification 54-095768 describes a process for the indirect preparation of an enriched β-casein fraction by removal of most of the α_s and κ-casein fractions. The process uses a caseinate solution as the starting material. The process uses a differential precipitation procedure.

Japanese patent specifications 59-091848 and 59-091849 address the total fractionation of whole casein. In the first specification, the preparation of a fraction containing α_s- and β-casein is described. The isolation of the β-casein from the α_s/β-casein fraction is the subject of the second patent. The isolation of the β-casein involves calcium precipitation of the α_s-casein and separation by centrifugation.

Japanese patent specifications 47 034936 and 55 16616 address the manufacture of human milk substitutes by altering the composition and/or character of the caseins in bovine milk. Specification 47 034936 discloses three methods for the isolation of β-casein enriched fractions. Each method is dependent on the removal of α_s-caseins. Specification 55 16616 discloses a process for isolating β-casein from desalted skim milk or a sodium caseinate solution. The process utilises a calcium precipitation procedure.

French Patent Specification 2,592,769 describes the production of β-casein enriched fraction and a β-casein depleted fraction by either the treatment of milk with a calcium complexing agent or the treatment of a caseinate solution with an agent which polymerises all the casein, adjusting the temperature to 0 to 7°C and subjecting the product to microfiltration using an inorganic membrane and tangential flow. The microfiltrate material is enriched in β-casein while the retentate material is depleted. Microfiltrate flow rates at such cold temperatures are very low making it difficult to justify such a process at an industrial level.

WO 92/00017 describes a process for extracting β-casein from a rennet casein suspension or solution. The process involves acidifying the suspension or solution to a pH of about 4 to 5 and cooling the solution to -2 to 10°C. Two phases are formed; a liquid phase containing β-casein and a solid phase which is depleted of substantially all of its β-casein. This solid phase no longer resembles the original rennet casein. The two phases are

physically separated. The process of the present invention, unlike the process of WO 92/00017, strips only a small proportion of the total β-casein from the casein curd.

United States Patent 5,169,606 described a process of cooling bovine milk to a temperature sufficient to dissociate β-casein to equilibrium and filtering the cooled milk through a microfilter to produce a humanized bovine milk product having an enriched β-casein content. The specification gives no indication as to what is done with the retentate by product of the microfiltration. Further, it is an object of this invention to produce a product containing a mixture of whey proteins and β-casein.

The Japanese Patent Specification 5,146,258 (91-337,701) describes a process for the isolation of β-casein using a combination of precipitation and membrane technologies. In the process a three stage procedure is used for isolating β-casein. Skim milk is first acidified to a pH of 3.5 or less and cooled. In the second step the pH is readjusted to 4.2 -4.4 to effect the precipitation of the α_s-casein fraction. A subsequent membrane filtration step in the cold (third stage) is used to effect the separation of β-casein. As in the method of WO92/00017 the remaining (β-casein depleted) casein is substantially altered in character and its commercial usefulness is no longer preserved.

It is desirable to provide a process for the extraction of β-casein from casein curd or powder which can be applied industrially and which produces economically viable quantities of β-casein without substantially altering the character or usefulness of the remaining casein. It is also desirable to provide a process which does not strip all or a substantial portion of the β-casein from the casein to produce two potentially useful products and avoids production of any waste by-products.

It is an object of the present invention to go some way towards achieving the above desiderata or at least provide the public with a useful choice.

DISCLOSURE OF THE INVENTION

Accordingly in a first aspect the present invention may be said broadly to consist in a process for extracting a β-casein enriched product and a β-casein depleted product from a casein feedstock including milk protein co-precipitate, said process comprising the steps of:

a) forming a slurry of said casein feedstock and cold water,

b) holding said slurry at a cold temperature and a pH between 5.0 and 8.0 for a rennet casein feedstock or a pH between 3.5 and 8.0 for other casein feedstock for a suitable period of time to effect extraction of β-casein, and

c) separating said slurry into two phases to obtain a solid phase which is β-casein depleted and a liquid phase which is β-casein enriched.

Preferably said casein feedstock is a wet casein curd or a milk protein powder containing casein.

More preferably said casein feedstock is mineral acid or lactic acid casein, milk protein isolate (TMP™ base), rennet casein, milk protein co-precipitate, casein co-precipitate, whole casein precipitated by calcium or other casein curd which contains β-casein.

Preferably said casein feedstock is a curd or powder of rennet casein.

Preferably said casein is fresh wet curd having a moisture content of between 45 and 75% w/w.

Preferably said fresh wet curd is subjected to wet milling to reduce the curd particle sizes to 1 mm diameter or less.

Alternatively said casein feedstock is a curd or powder of acid casein.

Preferably the concentration of casein in said slurry is 1-25% w/w.

More preferably the concentration of casein in said slurry is 6-15% w/w.

Preferably in step (b) said slurry is held at a pH of between 6.6 and 7.2 when rennet casein is used.

Preferably said slurry is held for between 0.1 and 30 hours at -10°C to 14°C.

More preferably said slurry is held for between 4 and 30 hours at 1 to 8°C.

More preferably said slurry is held for between 10 and 16 hours.

More preferably said slurry is held between 2 and 6°C.

Preferably said feedstock is casein curd and said process includes the preliminary step of extracting said casein curd from milk.

In one alternative said process is conducted as a batch process.

In a second alternative said process is conducted as a continuous process.

Preferably said β-casein depleted solid phase from step c) is subjected to a second extraction comprising said steps a) to c).

Preferably said β-casein depleted solid phase from step c) of said second extraction is subjected to a third extraction comprising said steps a) to c).

Preferably said process includes the further step of isolating β-casein enriched product from said liquid phase.

Preferably said process includes the further step of drying said isolated β-casein enriched product.

Preferably said step of isolating the said β-casein enriched product comprises steps of:

i) where necessary, acidifying said separated liquid phase,
ii) heating said liquid phase to effect precipitation of a β-casein curd, and
iii) isolating said β-casein curd.

Preferably, when said casein feedstock is mineral acid or lactic acid casein, milk protein isolate (TMP™ base), casein precipitated from milk by addition of calcium ions, or milk protein co-precipitate, said β-casein enriched liquid phase is concentrated prior to said step i).

Preferably said preconcentrating step comprises evaporation, ion exchange or membrane filtration of said liquid phase.

Preferably step (i) comprises adjusting the pH of said liquid phase to between 4.5 and 5.2.

Preferably step (ii) comprises heating said liquid phase to approximately 2 to 65°C for a period of between 0.6 seconds to 4 hours.

Alternatively step (ii) comprises heating said liquid phase to 25 to 45°C.

Alternatively said liquid phase is heated for 0.2 to 90 minutes.

Preferably an aqueous solution of sodium chloride is added in a concentration of up to 4.0% w/w is added to said slurry of step a).

Preferably step (iii) comprises isolating said β-casein curd by decanter separation, filter press, membrane filtration, clarifiers, screens or the like.

In one alternative said step of isolating β-casein from said liquid phase from step c) above comprises subjecting said liquid phase to a concentrating step followed by spray drying.

Preferably said concentrating step comprises evaporation, ion exchange or membrane filtration of said liquid phase with pH adjustment when required.

Preferably said process includes the further step of drying said β-casein depleted solid phase.

In a further aspect the present invention may broadly be said to consist in a β-casein enriched product when produced by the above identified process.

Preferably said β-casein enriched product contains 40 to 95% β-casein.

In a further aspect the present invention may broadly be said to consist in a β-casein depleted casein product when produced by the above identified process.

In a still further aspect the present invention may broadly be said to consist in a food, pharmaceutical, plastics or other non-food product including said β-casein enriched product and/or said β-casein depleted product.

Preferably said product is an infant food product.

This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

The invention consists in the foregoing and also envisages constructions of which the following gives examples.

BRIEF DESCRIPTION OF THE DRAWINGS

A preferred embodiment of the invention will now be described with reference to the examples and drawings in which:

Figure 1 is a schematic representation of the process of the invention including the optional preliminary step of forming a casein curd.

Figure 2 represents urea-PAGE analysis of samples from the investigation described in Example I. All samples were analysed in duplicate. The lanes are: 1 = a mixture of α_s-, β- and κ-casein standards; 2,3 = Skim milk used in the experiment; and 4,5,6 and 7 = β-extract recovered from the slurry at the end of the cold 19 hour extraction.

Figure 3 represents the urea-PAGE analysis of the dry-products obtained in example II. Lane 1 = a mixture of α_s- and β-casein standards; 2 and 3 pre-extraction rennet curd; 4 and 5 β-casein depleted casein curd; 6 and 7 β-casein enriched product.

Figure 4 represents a densitometer trace of a β-casein enriched product sample after electrophoresis on the urea-PAGE gel. (Refer lane 7 on Figure 3).

Figure 5 represents the urea-PAGE analysis of samples obtained from example III. Lane 1 = skim milk; 2 = β-extract (6h); 3 = β-extract (19h); 5 = rennet casein before-extraction; 6 extracted rennet casein; 7 = β-casein enriched product.

Figure 6 is a schematic diagram of the process of the large scale pilot plant extraction of β-casein enriched product described in example IV.

Figure 7 represents the urea-PAGE analysis of the products from example V. The lanes are: 1 and 2 = standard New Zealand Alacid 720™ acid casein used for the extraction; 3, 4, 5, 6, 7 and 8 were the extracts recovered from the 0%, 0.25%. 0.5%, 1.0%, 2.0%, and 4.0% w/v salt extractions (respectively); 9 = β-casein standard.

Figure 8 represents the urea-PAGE analysis of the products from example VI. The lanes are: 1 and 2 = whole casein used for the extraction; 3, 4, 5, 6, 7 and 8 are the extracts recovered from the slurries which contained 0%, 0.25%. 0.5%, 1.0%, 2.0%, and 4.0% salt (respectively); 9 = β-casein standard.

Figure 9 represents the urea-PAGE analysis of the products from example VII. The lanes are: 1 and 2 = casein coprecipitate used as the starting material for the extraction; 3 = β-casein enriched product from the water extraction; 4 = water extracted casein coprecipitate; 5 = β-casein enriched product obtained from the saline extraction; 6 = salt extracted casein coprecipitate; 7, 8, and 9 were the α_s-, β- and κ-casein standards.

Figure 10 represents the urea-PAGE analysis of the products from example VIII. The lanes are : 1 and 2 = standard New Zealand sulphuric acid casein (Alacid™ 750) used for the extraction; 3, 4, 5, 6, 7 and 8 are the extracts recovered from the 0%, 0.25%. 0.5%, 1.0%, 2.0%, and 4.0% w/v salt extractions (respectively); 9 = β-casein standard.

Figure 11 represents the urea-PAGE analysis of products from example IX. The lanes are: 1 = a mixture of α_s- and β-casein standards; 2 and 3 = pre-extraction acid casein; 4 and 5 = extracted acid casein; 6 and 7 = β-casein enriched product.

Figure 12 represents the urea-PAGE analysis of the products from example X. The lanes are: 1 and 2 = water extracted New Zealand rennet casein Alaren™ 771; 3 = β-casein enriched product from the water extraction of the same rennet casein; 4 = the rennet casein sample used for the experiment; 5 = β-casein enriched product obtained from the saline extraction; 6 = salt extracted rennet casein sample; 7, 8, and 9 were the α_s-, β- and κ-casein standards.

MODES OF CARRYING OUT THE INVENTION

Rennet casein curd may be prepared from mammalian milk, preferably cows milk, using known methods (such as those described by Weal and Southward, 1974; L L Muller, 1971; Southward and Walker, 1980; Southward and Walker, 1982; Southward, 1985; Mulvihill, 1989). A preferred method is that described in Weal and Southward, 1974 and Southward and Walker, 1980.

In the process of the invention as shown in figure 1 fresh washed, dewatered casein curd prepared from skim milk by a known method, which typically contains 40 to 75% (w/w) moisture is added to water to form a slurry. Optionally, the curd may be wet milled to reduce curd particle sizes to 1 mm or less. The concentration of curd in the slurry is within the range 5-25% w/w, preferably between 6 and 15% w/w. Alternatively, acid casein curds, the milk protein isolate (TMP™ base) curd or the milk protein

co-precipitate may be used in the process of the invention instead of rennet casein curd with appropriate adjustment of the reaction conditions. The water to which the rennet casein curd is added is at a temperature of between 1 and 30°C, preferably between 2 and 5°C. Alternatively, the water may be added at ambient temperature and then cooled to the desired temperature. The rennet casein curd slurry is held for 3 to 30 hours, preferably for 12 to 16 hours at 1 to 12°C, preferably 2 to 5°C. It is preferred that the slurry is gently agitated during this cold extraction. In a commercial process it is envisaged that the curd may be extracted either as detailed above or for periods between 0.1 minute and 16 hours at the given or lower temperatures. The pH of a slurry of rennet casein is adjusted to be above pH 5.0 during the cold extraction, preferably between pH 6.8 and 7.2 which is the natural pH of the system. Where the casein and feedstock is other than rennet casein, for example acid casein curd, the pH is adjusted to 3.0 to 8.0. A soluble β-casein enriched fraction and a solid β-casein depleted curd fraction are obtained in either case.

The curd solids and soluble β-casein fraction may be separated using a screen, decanter, clarifier, filter press, membrane filtration, centrifugation or any other suitable method. An aqueous β-extract and a β-casein depleted rennet casein curd solid fraction are obtained.

The β-casein depleted rennet casein curd fraction recovered by decanter centrifugation (or any other suitable method) is dried in the usual way.

The β-casein may be recovered from the aqueous β-casein extract by acidifying the β-extract after warming. Preferably the pH is adjusted to 4.5 - 5.2, most preferably 4.6 - 4.9. The pH may be adjusted as the β-extract comes off the mechanical centrifuge or alternatively within a holding vat.

The pH is adjusted by the addition of an organic or inorganic acid or a mixture thereof. Suitable organic acids include but are not limited to acetic acid, lactic acid, citric acid, tartaric acid or a mixture of any two or more of these. Suitable inorganic acids include but are not limited to hydrochloric acid, sulphuric acid, phosphoric acid or mixtures thereof.

Prior to acidification the β-casein may be concentrated or desalted by any combination of by evaporation, membrane filtration or the like. In addition, or alternatively, the mineral composition of the extract can be altered by ultrafiltration and/or diafiltration of the extract. The diafiltered mineral reduced or mineral adjusted product can in addition, or alternatively, be recovered by spray drying. Altering the mineral composition

of the extract produces an end product which is a micellar (calcium phosphate) proteinate or a mineral caseinate form eg. ammonium, sodium, potassium, calcium, magnesium or an alkaline earth, and monovalent or divalent or any mixture thereof. In addition, the β-casein fraction can be dephosphorylated by known methods at this stage. In addition hydrolysis may be used to produce modified forms or peptides of β-casein.

Alternatively, the β-casein extract is heated to a temperature of 2 to 65°C, preferably 25 to 45°C for 0.1 minute to 4 hours, preferably 0.2 to 0.5 minutes. The β-casein enriched curd obtained may be separated from solution by decanter separation, a filter press, membrane filtration, clarifiers and/or the use of a screen or any other suitable method.

In another alternative illustrated in figure 1 the β-extract can be concentrated by, for example, evaporation, ion exchange or membrane filtration, followed by spray drying to obtain the β-casein enriched product.

The β-casein enriched curd and β-casein depleted casein curd obtained by the process of the invention may be dried by any suitable method, for example freeze drying, ring drying, roller drying and fluid bed drying for the extracted rennet casein and the acid form of β-casein enriched product. The proteinate or micellar forms of the β-casein enriched product may be dried additionally by spray drying.

Using this process it is possible to extract β-casein enriched product in an amount between 0.1 and 10%, typically 3 to 6% w/w of the total casein present in the milk or casein curd used as the feed material. The purity of the β-casein enriched product is between 40% to 95% preferably between 60 to 85%.

The β-casein depleted casein product remaining is between 90 to 99% preferably 94 to 98% of the total casein present in the casein feedstock used as the starting material. The β-casein depleted product was very similar in its properties, including chemical composition, as the casein starting material. Known industrial uses for casein are discussed in reference 5.

Example I

Laboratory scale demonstration of the feasibility of producing β-casein enriched product from rennet casein curd

Fresh skim milk (2.5 L) was warmed to 30°C and calf rennet was added (0.22ml/L of milk). After 30 minutes at 30°C the rennet casein coagulum which formed was heated

in a water bath at 55°C for 5 minutes. The resulting rennet casein curd was recovered by straining through muslin cloth. The curd solids were washed batchwise with three lots of water (approximately 1 L) at 60°C and finally with water at 18°C. The washed rennet casein curd was suspended in 800 ml water. The suspension was held at 4°C overnight (19h) to effect the extraction of 6-casein enriched product from the rennet casein curd. The 6-casein depleted casein curd solids in the slurry were separated (as before) and the aqueous extract (6-extract) was analysed by urea polyacrylamide gel electrophoresis (urea-PAGE) as described by Andrews (Journal of Dairy Research 50: 45-55). The results, given in Figure 2 show that the aqueous extract contained 6-casein as the predominant protein.

Example II

Small scale pilot plant extraction of 6-casein enriched product from rennet casein curd.

Fresh skim milk (800 L) was warmed to 30°C and calf rennet (160ml) was added with mixing. After 30 minutes at 30°C the soft protein coagulum which formed was gently heated to 55°C. The resulting casein curd was dewheyed on a screen and washed in the standard way. (ref 1,3). The washed curd was dewatered with the aid of a decanter. The rennet casein recovered was approximately 48 kg.

The fresh dewatered curd was slurried with 480L of water and chilled to 2.7°C. The slurry was held under gentle agitation at this temperature for 23 hours. The slurry was then pumped to a screen to separate the curd from the aqueous 6-extract. The 6-extract so recovered (approximately 400 L) was acidified manually to pH 4.4 with dilute sulphuric acid and heated to 55°C. The 6-casein solids which formed as the acid curd were collected (manually) by filtration through 50μm GAF bags. The 6-casein enriched curd so recovered was freeze dried to obtain 0.59 kg of 6-casein enriched product.

Samples of both the pre-extraction rennet casein curd and the 6-casein depleted casein curd were dried for analysis. The results of the urea-PAGE analysis are shown in Figure 3. The results show that the 6-casein depleted casein curd recovered at the end of the extraction is similar in composition to the unextracted control with respect to the gross composition of the α_s- and 6-casein fractions. The removal of the 6-casein fraction from the 6-casein depleted casein curd was small as judged by the low yield of product (approximately 2.7% w/w of the total casein protein). The minimal extraction of the 6-casein from the 6-casein depleted casein curd was confirmed by the densitometric analysis. This analysis shows that the ratio of the α_s- to 6-casein fractions in the 6-casein depleted casein curd was only slightly changed after the extraction. The 6-casein

enriched product recovered from the aqueous β-extract was shown to contain essentially β-casein (Figure 3). Densitometric analysis of the urea-PAGE gel revealed the β-casein enriched product sample to be of a purity of approximately 80% (Figure 4).

Example III

Pilot plant production of β-casein enriched product

Referring again to figure 1, approximately 900 L of fresh skim milk was set with calf rennet (200 ml rennet) at 27°C. After heating the coagulum was separated by screen dewheying. The dewheyed curd was washed in the standard way followed by decanter dewatering.

The dewatered rennet casein curd (66.8 kg) combined with chilled water (3°C) was subjected to wet milling to generate curd particles of 1mm or less in diameter before being placed in a silo. The mixture was continuously stirred and held overnight (19 h).

The slurry mixture was then pumped to a decanter for separation of the β-casein depleted casein curd solids from the β-extract. The casein curd solids recovered were dried in the usual way (21 kg of β-casein depleted product was obtained).

The β-extract recovered from the cold separation step (680 L) was placed in a silo and acidified to pH 4.7. The acidified mixture was then heated to 36-38°C prior to separation in the decanter for recovery of β-casein enriched curd (2.49 kg) and ring drying (1 kg dried β-casein enriched product in the form of a powder recovered).

The gross chemical composition of the pre-extraction rennet casein, the β-casein depleted casein product and the β-product are given in Table 1 below. The results show that the rennet casein composition is essentially unchanged from that of the pre-extraction rennet casein in respect to ash, calcium, lactose, moisture and total nitrogen (TN) contents after the extraction.

Urea-PAGE analysis (Figure 5) showed that the β-extract contained β-casein as the major component and that other casein fractions were not present.

TABLE I

The gross chemical composition of the rennet caseins and β-product of example III.

Sample	Ash (% w/w)	Calcium^{2+} (mM/kg)	Fat (% w/w)	Lactose (% w/w)	Moisture (% w.w)	TN (% w.w)
Pre-extraction rennet casein	8.56	788	0.50	0.23	7.33	13.41
6-casein depleted casein product	8.78	720	0.41	0.20	6.85	13.52
6-casein enriched product	1.44	ND+	0.82	ND+	5.84	14.64

ND+ = not determined

Example IV

Large Scale Pilot Plant Extraction of 6-casein enriched product

Referring to figure 6 of the drawings fresh skim milk (13850 L) was treated with calf rennet and processed under standard conditions for the manufacture of casein curd. Wet casein curd (881 kg) was conveyed via a holding tank to a colloid mill where the curd was wet milled to particles of approximately 1.02 mm diameter. Approximately 3200 L of chilled water was used to complete the wet milling of the curd. The milled rennet casein curd slurry was pumped to a silo (at approximately 6°C) and cold water was added (final volume of 11430 L approx). The slurry was held cold and agitated gently for the next 17 h (end temperature was 3.6°C).

The 6-casein depleted casein curd solids were recovered by a decanter and dried in the usual way.

The liquid 6-casein enriched extract at 30°C from the decanter was acidified (in-line) with 4% w/w H_2SO_4 en route to a second decanter for the recovery of the 6-casein enriched product. The end pH to which the extract was acidified was 4.7. The resulting

β-casein enriched curd was dried in a ring drier. 13.0 Kg of dried β-casein enriched product was obtained.

Example V

Extraction of β-casein enriched product from Standard New Zealand lactic acid casein

Samples (1.00 +/- 0.05) g of New Zealand lactic casein (Alacid™ 720) were suspended in 10 mL of water containing between 0 and 4 % w/w sodium chloride. The slurries were mixed by inversion for 20 hours at 3.7°C and then centrifuged at 13,000g to separate the extract from the solids. Total protein in the extract was estimated by determining the absorbance at 280 nm and using β-casein as the reference protein for calibration. The results showed that the extracts obtained from the 0%, 0.25%, 0.5%, 1.0%, 2.0% and 4.0% w/v salt extractions contained 10 mg, 32 mg, 50 mg, 59 mg, 71 mg and 62 mg protein, respectively. When the extracts were analyzed by urea-PAGE (Figure 7) all the extracts were shown to contain β-casein as the predominant protein. Densitometric analysis of the urea-PAGE gel showed that the β-casein in the extracts ranged in purity between 51% and 76% depending on the level of salt added for the extraction.

Example VI

Extraction of β-casein enriched product from whole casein precipitated with calcium

Skim milk was warmed to 55°C and calcium was added to 3% w/v to precipitate the caseins. The casein precipitate was washed to remove whey proteins and dried in the usual way.

Samples of the dried casein (1.00 +/- 0.05 g) were suspended in water (final volume 10 mL) containing the same range of sodium chloride concentrations described in Example

V. The slurries were extracted as described for Example V and the soluble protein extracts which were recovered were analyzed in a similar manner. The results showed that the extracts obtained from the 0%, 0.25%, 0.5%, 1.0%, 2.0% and 4.0% w/v salt extractions contained 40 mg, 45 mg, 55mg, 53 mg, 48 mg, and 30.5 mg protein, respectively. The results of the urea-PAGE analysis in Figure 8 showed that all the extracts contained β-casein as the predominant protein. Densitometric analysis of the urea-PAGE gel showed that the β-casein in the extracts ranged in purity between 42% and 53% depending on the level of salt added at the start of the extraction.

Example VII

Extraction of β-casein enriched product from New Zealand milk protein isolate

A sample (2.05 kg) of New Zealand milk protein isolate (TMP™ 1000 base) was slurried in 8.05 kg cold water. A second sample (2.01 kg) was slurried in water (8.07 kg) which contained 106 g of sodium chloride (saline extraction). Both slurries were agitated with the aid of an overhead stirrer at 2.4°C for 18 hours after which the solids were separated from the extract by filtering the slurry sequentially through 125 micron and 25 micron GAF bags. The extracted casein solids were freeze dried (recovered 1.74 kg and 1.49 kg for the coprecipitate samples extracted with water and the saline solution, respectively). The soluble extracts (4.228 kg and 3.49 kg from the samples extracted with water and the saline solution, respectively) were heated to (and held at) 60°C under agitation for between 30 and 90 min. The β-casein enriched curd solids which formed were recovered by filtering through the 25 micron GAF bag. The β-casein enriched curd solids were freeze dried (3.24 g and 100.3 g β-casein enriched product were obtained from the water and the saline extractions, respectively). When the dried products were analysed by urea-PAGE (Figure 9) the results showed that the products recovered from the water and saline extracts were enriched 1.54 and 1.23 fold respectively in β-casein.

Example VIII

Extraction of β-casein enriched product from whole sulphuric acid casein

Samples (1.00 +/- 0.05) g of New Zealand sulphuric acid casein (Alacid™ 750) were suspended in 10 mL of water containing between 0 and 4 % w/w sodium chloride as described for example V above. The slurries were mixed by inversion for 22 hours at 3.7 (+/- 0.5)°C and then centrifuged at 13,000g to separate the extract from the solids. Total protein in the extract was estimated by determining the absorbance at 280 nm as described before (see example V). The results showed that the extracts obtained from the extractions with 0%, 0.25%, 0.5%, 1.0%, 2.0% and 4.0% w/v salt contained 10 mg, 32 mg, 56 mg, 80 mg, 88 mg and 66 mg protein, respectively. Urea-PAGE analysis (Figure 10) showed that all the extracts contained β-casein as the predominant protein. Densitometric analysis of the urea-PAGE gel showed that the β-casein in the extracts ranged in purity between 57% and 86% depending on the level of salt added for the extraction.

Example IX

Extraction of β-casein enriched product from fresh acid casein curd

Fresh decanter dewatered sulphuric acid casein curd was prepared as follows: 800 L of pasteurised skim milk was cooled to 21°C and 1.0 N sulphuric acid was injected in-line to obtain a pH of 4.5 to 4.6. The coagulum was then heated to 49°C for cooking followed by standard acidulation and screen dewheying procedures. The dewheyed curd solids were subjected to the usual washes and dewatered in a decanter. (Approximately 43.03 kg of fresh acid casein curd was recovered).

The fresh acid casein curd was agitated in 480 L of water at 3.5°C for 19½ hours. The curd solids were separated from the aqueous β-casein extract (550 L) by the use of a decanter. The acid casein curd was dried in the usual way.

The β-casein extract (550 L) recovered from the cold separation step was heated in-line by direct steam injection and fed to the decanter at 38°C for the recovery of the β-casein curd. The wet β-casein curd was dried. The dry β-casein product recovered weighed 0.225 kg.

Urea-PAGE analysis (Figure 11) showed that the β-casein content of 54% for the product represented a 1.5 fold enrichment of β-casein when compared to the original acid casein.

The gross chemical composition of the pre-extraction mineral acid casein, the extracted mineral acid casein and the β-casein enriched product are given in Table 2 below. The results show that the β-casein depleted mineral acid casein was similar to the original casein in composition, with the exception that the level of fat was lowered after the extraction.

Table 2

The gross chemical composition of the acid casein feedstock and β-casein enriched product from example IX.

Sample	TN (% w/w)	Moisture (% w/w)	Fat (% w/w)	Ash (% w.w)
Pre-extraction acid casein	14.1	8.6	1.38	1.8
β-casein depleted acid casein	13.95	9.9	0.70	1.7
β-casein enriched product	14.0	7.6	2.14	1.5

Example X

Extraction of β-casein from rennet casein

A sample of New Zealand rennet casein (Alaren™ 771) (700 g) was slurried in 4.00 kg cold water. A second sample (700 g) was suspended in 4.02 kg cold water which contained 80.4 g of sodium chloride (saline extraction). Both slurries were agitated for 17 hours at 2.4°C after which the solids were separated from the extract by filtering the slurry (sequentially) through a 125 micron and a 25 micron GAF bag. The extracted casein solids were freeze dried. The soluble extracts were acidified with 0.05 M sulphuric acid to pH 4.65 and heated to 40°C under agitation. The β-casein curd solids which formed were recovered by filtering through the 25 micron GAF bag and then freeze dried (recovering 58.9 g and 13.24 g β-casein enriched product from the water and the saline extractions, respectively). When the dried products were analysed by urea-PAGE (Figure 12) the results showed that the protein recovered from the water and saline extracts were enriched 1.7 and 1.2 fold respectively in β-casein.

ADVANTAGES

At least in the preferred form the invention has the following advantages:

1. The process may be readily introduced into the standard method for the commercial manufacture of casein.

2. The process results in the production of two potentially useful fractions. Because the process only strips a small fraction of the β-casein present, the β-casein depleted casein is a useful product in addition to the β-casein product. Thus, there is substantially reduced waste.

3. The process produces β-casein product of high quality on an industrial scale in economically viable quantities.

4. The process of the present invention does not rely on the use of sophisticated or additional equipment beyond that which is commonly found in facilities where casein is manufactured to modern day standards.

5. The process uses processing steps that are consistent with food industry practice and does not also require unusual processing aids or additives.

References:

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2. Manufacture and use of co-precipitates; A review. L L Muller, Dairy Science Abstracts, 33 (9), pp 659-674 (1971).

3. The manufacture and industrial uses of casein. C R Southward & N J Walker, *New Zealand Journal of Dairy Science and Technology* 15 : 201-217 (1980).

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5. Utilisation of milk components: Casein. C R Southward. In *Modern Dairy Technology, Vol L Advances in Milk Processing* (Ed R K Robinson). Elsevier Applied Science Publishers, London, 1985, pp 317-368.

6. Casein and Caseinates: Manufacture. D M Mulvihill. In *Developments in Dairy Chemistry*, Vol. 4 (Ed P F Fox). Elsevier Applied Science Publishers, London, 1989, pp 97-130.

7. Proteins of Milk. R McK Whitney. In *Fundamentals of Dairy Chemistry*, third edition, chapter 3, 81-170, by N P Wong, R P Jenness, M Keeney and E M Marth (Eds). Van Nostrand Reinhold, New York.

CLAIMS:

1. A process for extracting a β-casein enriched product and a β-casein depleted product from a casein feedstock including milk protein co-precipitate, said process comprising the steps of:

a) forming a slurry of said casein feedstock and cold water,

b) holding said slurry at a cold temperature and a pH between 5.0 and 8.0 for a rennet casein feedstock or a pH between 3.5 and 8.0 for other casein feedstock for a suitable period of time to effect extraction of β-casein, and

c) separating said slurry into two phases to obtain a solid phase which is β-casein depleted and a liquid phase which is β-casein enriched.

2. A process according to claim 1 wherein said casein feedstock is a wet casein curd or a milk protein powder containing β-casein.

3. A process according to claim 1 or 2 wherein said casein feedstock is mineral acid or lactic acid casein, milk protein isolate, rennet casein, milk protein co-precipitate, casein co-precipitate, whole casein precipitated by calcium or other casein which contains β-casein.

4. A process according to claim 1, 2 or 3 wherein said casein feedstock is a curd or powder of rennet casein.

5. A process according to claim 1, 2 or 3 wherein said casein feedstock is a curd or powder of acid casein.

6. A process according to any one of the preceding claims wherein said casein is fresh wet curd having a moisture content of between 45 and 75% w/w.

7. A process according to claim 6 wherein said fresh wet curd is subjected to wet milling to reduce the curd particle sizes to 1mm diameter or less.

8. A process according to any one of the preceding claims wherein the concentration of casein in said slurry is 1-25% w/w.

9. A process according to claim 8 wherein the concentration of casein in said slurry is 6-15% w/w.

10. A process according to any one of claims 4, 6 and 7 wherein the casein feedstock is rennet casein and in step (b) said slurry is held at a pH of between 6.6 and 7.2.

11. A process according to any one of claims 5, 6 and 7 wherein the casein feedstock is other than rennet casein and in step (b) said slurry is held at a pH of between 4.5 and 6.0.

12. A process according to any one of the preceding claims wherein said slurry is held for between 0.1 and 30 hours at -10°C to 14°C.

13. A process according to claim 12 wherein said slurry is held for between 4 and 30 hours at 1 to 8°C.

14. A process according to claim 13 wherein said slurry is held for between 10 and 16 hours.

15. A process according to any one of claims 12 to 14 wherein said slurry is held between 2 and 6°C.

16. A process according to any one of the preceding claims wherein the feedstock is casein curd and which includes the preliminary step of extracting said casein curd from milk.

17. A process according to any one of the preceding claims which is conducted as a batch process.

18. A process according to any one of claims 1 to 16 which is conducted as a continuous process.

19. A process according to any one of the preceding claims wherein said β-casein depleted solid phase from step c) is subjected to a second extraction comprising said steps a) to c).

20. A process according to claim 19 wherein said β-casein depleted solid phase from step c) of said second extraction is subjected to a third extraction comprising said steps a) to c).

21. A process according to any one of the preceding claims which includes the further step of isolating β-casein enriched product from said liquid phase.

22. A process according to claim 21 which includes the further step of drying said isolated β-casein enriched product.

23. A process according to claim 21 or 22 wherein said step of isolating the said β-casein enriched product comprises the steps of:

i) where necessary, acidifying said separated liquid phase,

ii) heating said liquid phase to effect precipitation of a β-casein curd, and

iii) isolating said β-casein curd.

24. A process according to claim 23 wherein when said casein feedstock is mineral acid or lactic acid casein, milk protein isolate, casein precipitated from milk by addition of calcium ions, or milk protein co-precipitate, said β-casein enriched liquid phase is concentrated prior to said step i).

25. A process according to claim 23 wherein said preconcentrating step comprises evaporation, ion exchange or membrane filtration of said liquid phase.

26. A process according to any one of claims 23 to 25 wherein said step (i) comprises adjusting the pH of said liquid phase to between 4.5 and 5.2.

27. A process according to any one of claims 23 to 26 wherein said step (ii) comprises heating said liquid phase to approximately 2 to 65°C for a period of between 0.6 seconds to 4 hours.

28. A process according to claim 27 wherein said step (ii) comprises heating said liquid phase to 25 to 45°C.

29. A process according to claim 27 or 28 wherein said liquid phase is heated for 0.2 to 90 minutes.

30. A process according to any one of claims 23 to 29 wherein said step (iii) comprises isolating said β-casein enriched product by decanter separation, filter press, membrane filtration, clarifiers, screens or the like.

31. A process according to any one of the preceding claims which includes the additional step of adding an aqueous solution of sodium chloride in a concentration of up to 4.0% w/w to said slurry of step a).

32. A process according to claim 21 wherein said step of isolating β-casein enriched product from said liquid phase from step c) above comprises subjecting said liquid phase to a concentrating step followed by spray drying.

33. A process according to claim 32 wherein said concentrating step comprises evaporation, ion exchange or membrane filtration of said liquid phase with pH adjustment when required.

34. A process according to any one of the preceding claims which includes the further step of drying said β-casein depleted solid phase.

35. A β-casein enriched product when produced by the process of any one of the preceding claims.

36. A β-casein enriched product according to claim 34 containing 40 to 95% w/w β-casein.

37. A β-casein depleted casein product when produced by the process of any one of claims 1 to 34.

38. A food, pharmaceutical, plastics or other non-food product containing a β-casein enriched product according to claim 33 or 34.

39. A product according to claim 38 which is an infant food product.

40. A food, pharmaceutical, plastics or other non-food product containing a β-casein depleted product according to claim 37.

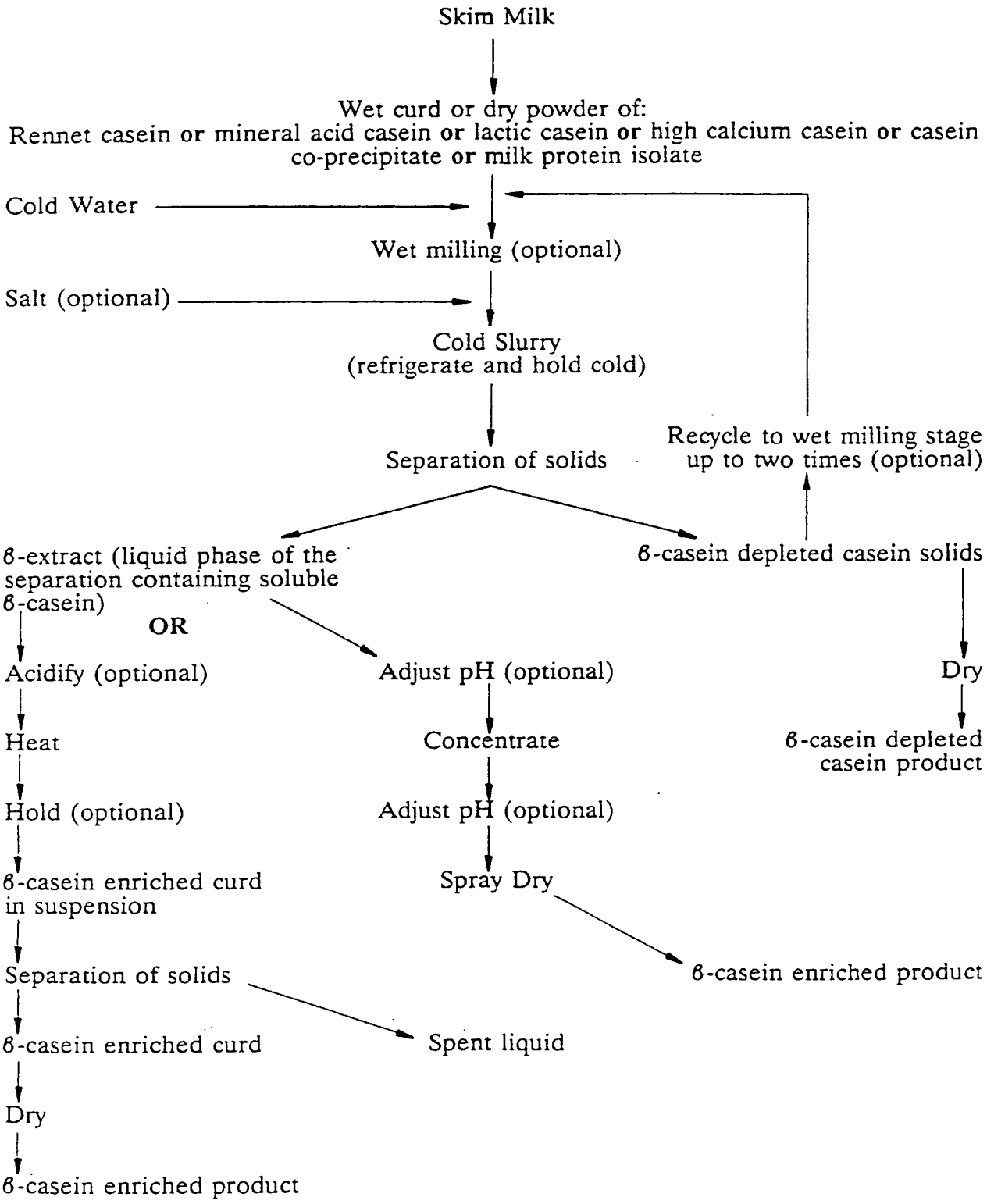
Skim Milk
Wet curd or dry powder of:
Rennet casein or mineral acid casein or lactic casein or high calcium casein or casein co-precipitate or milk protein isolate
Cold Water
Wet milling (optional)
Salt (optional)
Cold Slurry
(refrigerate and hold cold)
Separation of solids
Recycle to wet milling stage up to two times (optional)
β-extract (liquid phase of the separation containing soluble β-casein)
OR
β-casein depleted casein solids
Acidify (optional)
Adjust pH (optional)
Dry
Heat
Concentrate
β-casein depleted casein product
Hold (optional)
Adjust pH (optional)
β-casein enriched curd in suspension
Spray Dry
Separation of solids
β-casein enriched product
β-casein enriched curd
Spent liquid
Dry
β-casein enriched product

FIG 1

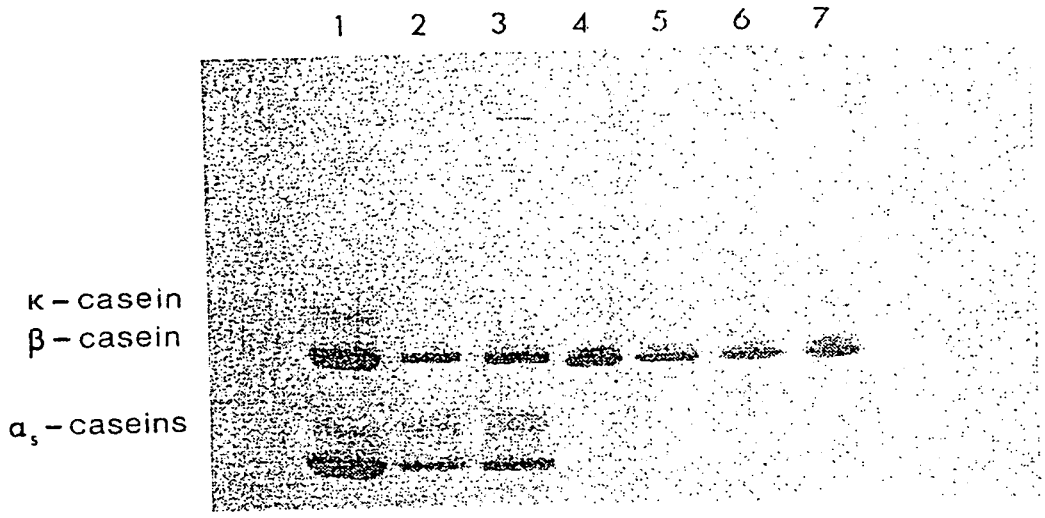

FIG 2

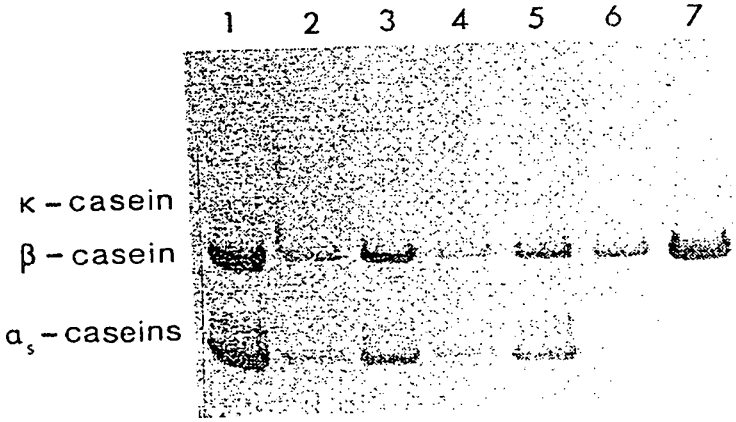

FIG 3

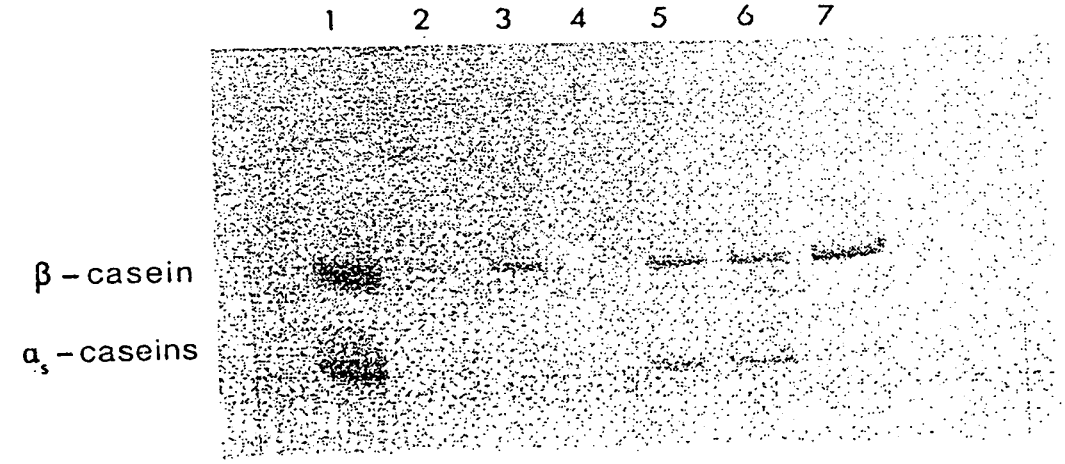

FIG 5

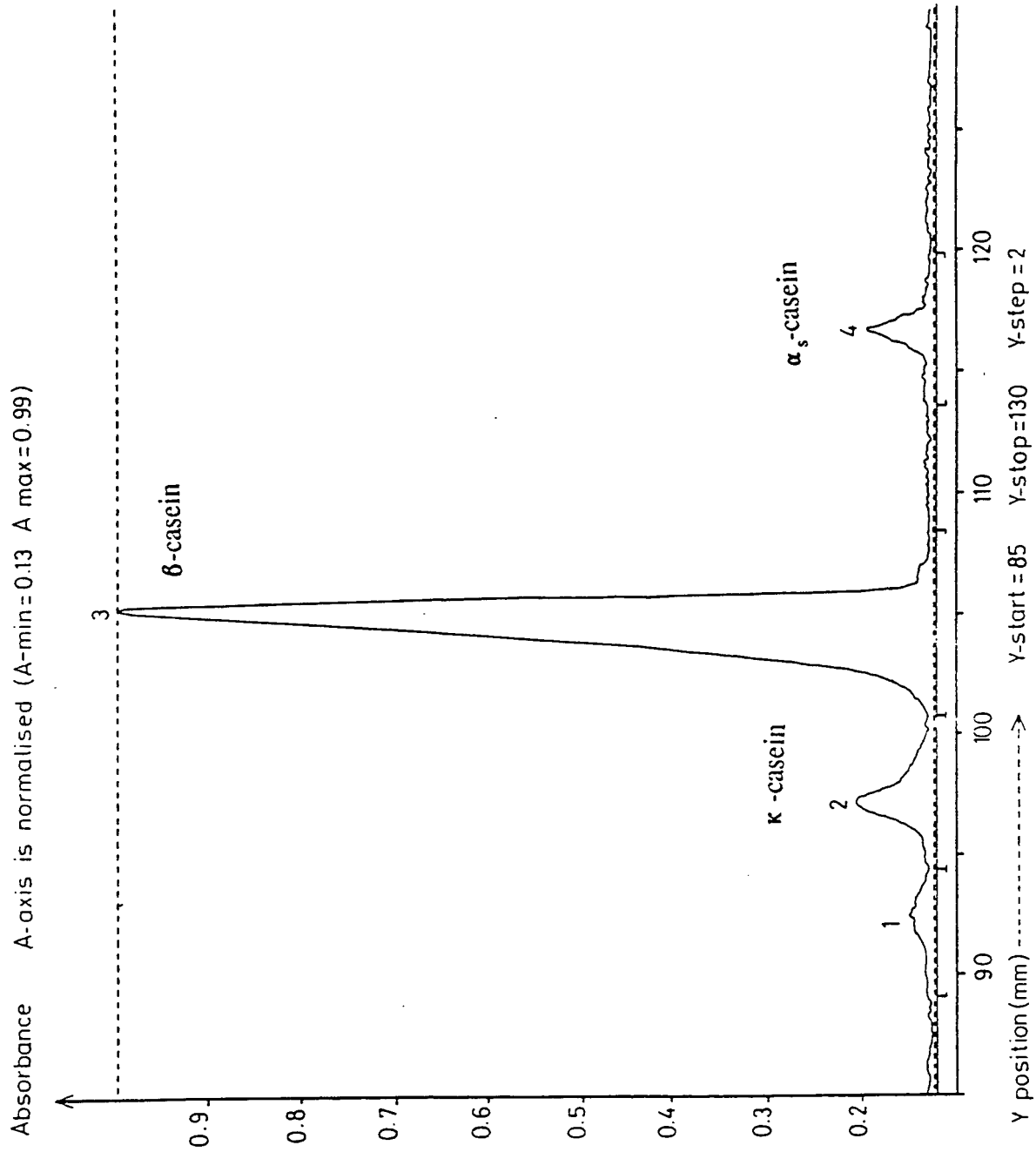
Absorbance
A-axis is normalised (A-min=0.13 A max=0.99)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
1
2
3
4
κ -casein
β-casein
α_s-casein
90
100
110
120
Y position (mm)
Y-start = 85
Y-stop=130
Y-step = 2

FIG 4

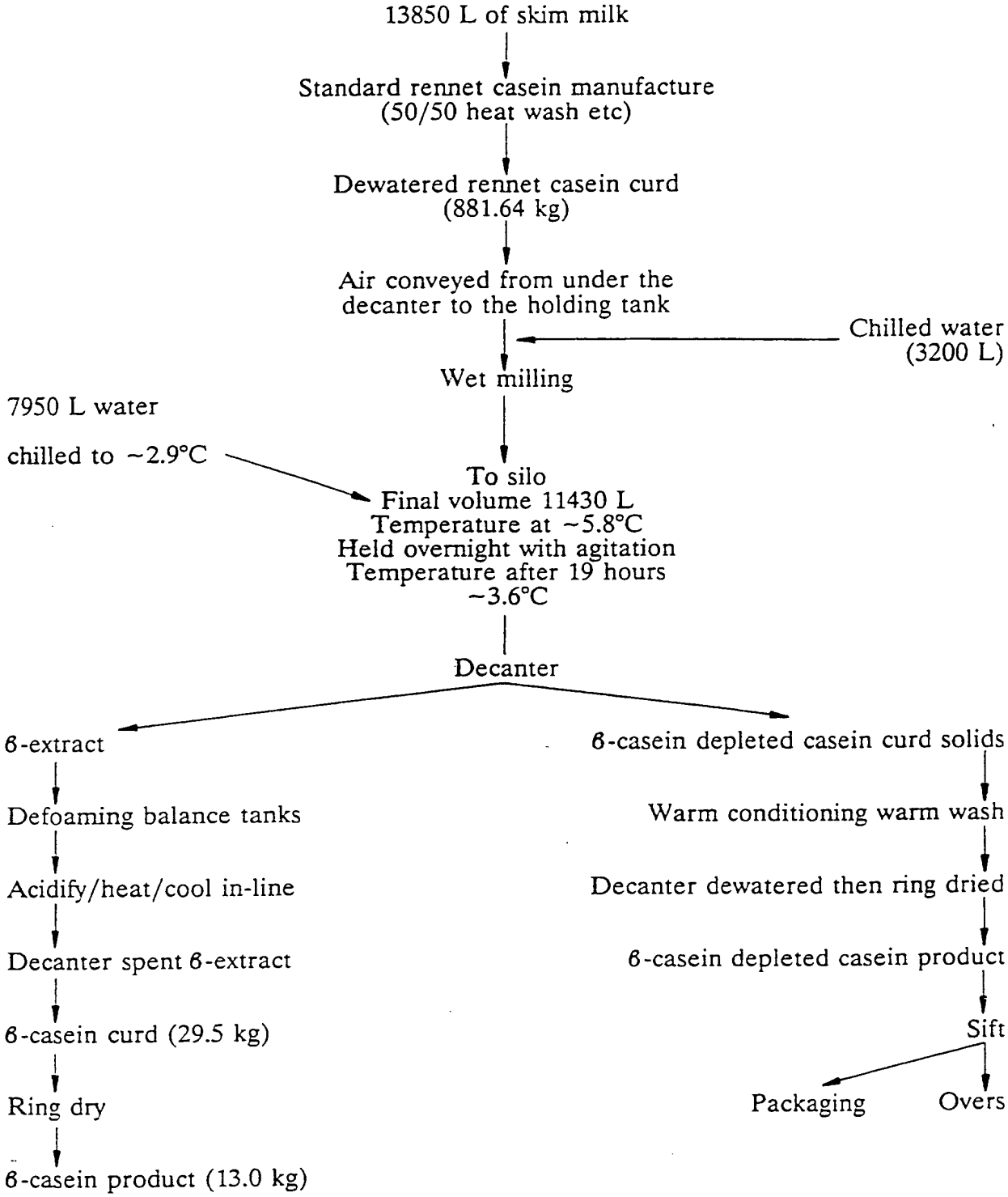
13850 L of skim milk
Standard rennet casein manufacture (50/50 heat wash etc)
Dewatered rennet casein curd (881.64 kg)
Air conveyed from under the decanter to the holding tank
Chilled water (3200 L)
Wet milling
7950 L water
chilled to ~2.9°C
To silo
Final volume 11430 L
Temperature at ~5.8°C
Held overnight with agitation
Temperature after 19 hours ~3.6°C
Decanter
β-extract
β-casein depleted casein curd solids
Defoaming balance tanks
Acidify/heat/cool in-line
Decanter spent β-extract
β-casein curd (29.5 kg)
Ring dry
β-casein product (13.0 kg)
Warm conditioning warm wash
Decanter dewatered then ring dried
β-casein depleted casein product
Sift
Packaging
Overs

FIG 6

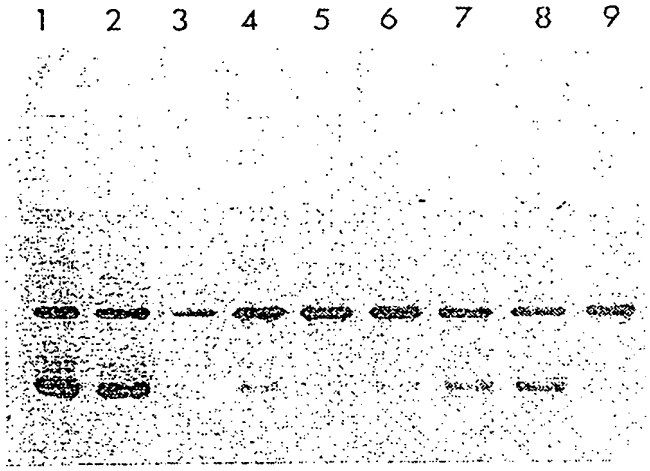

FIG 7

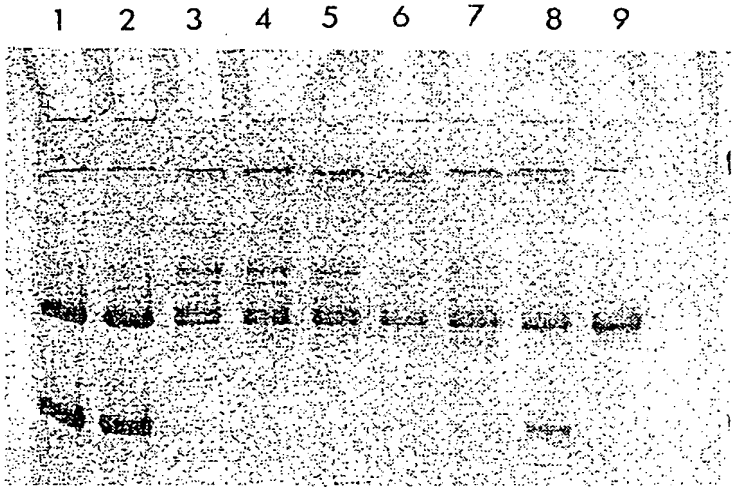

FIG 8

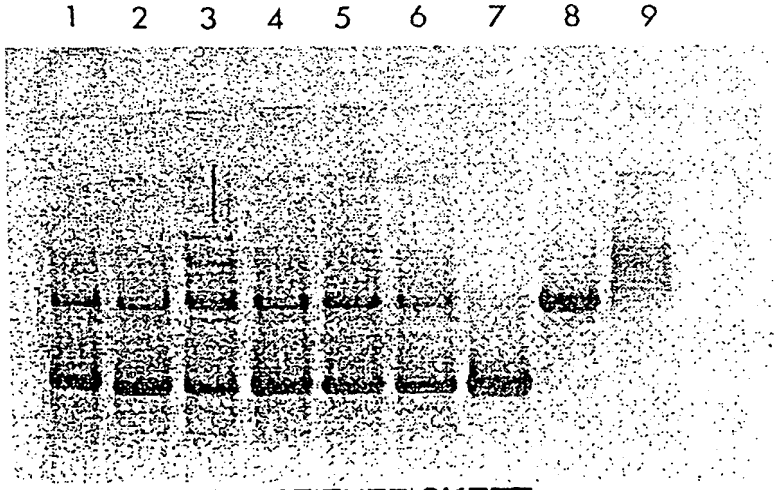

FIG 9

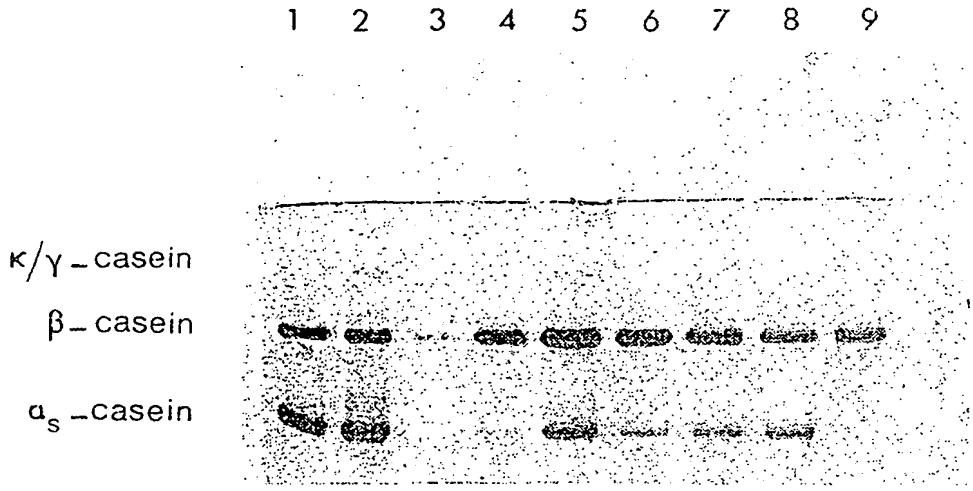

FIG 10

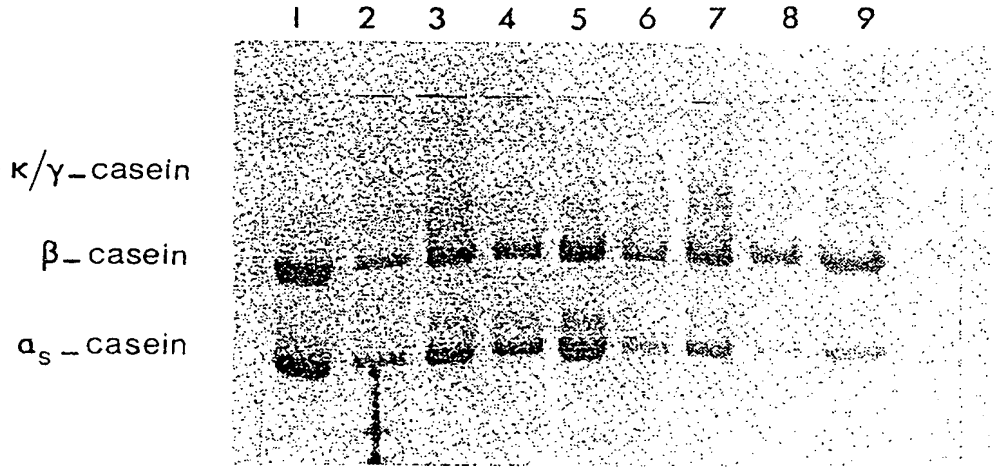

FIG 11

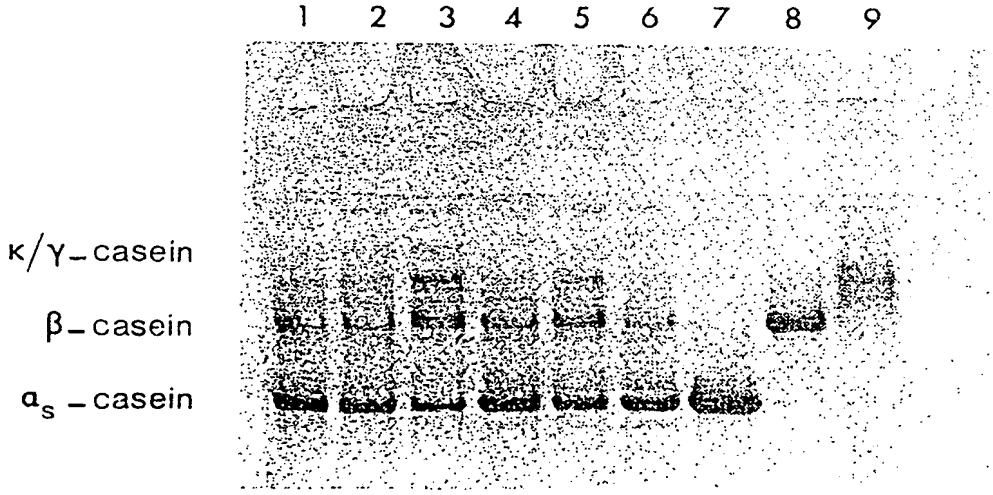

FIG 12

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] A23J 1/20, A61K 37/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : A23J 1/20, A61K 37/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU : IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X,Y	WO,A, 92/00017 (EURIAL) 9 January 1992 (09.01.92). See whole document.	1-40
Y	FR,A, 2592769 (INSTITUT NATIONAL DE LA BECHERCHE AGRONOMIQUE) 7 July 1987 (17.07.87). See whole document.	1-40
Y	Derwent Abstract Accession no. 656218/36, Class D13, JP,A, 54/95768 (SNOW BRAND MILK PRODUCTS) 11 January 1978 (11.01.78)	1-40

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 1993 (15.12.93)	21 DEC 1993 (21.12.93)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
Y	WO,A, 91/02539 (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE) 7 March 1991 (07.03.91). See whole document.	1-40

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 9200017	AU 80999/91, CA 2086006, EP 536290, FR 2663637, HU 9204126, NO 924967
FR 2592769	
JP 54095768	
WO 9102539	EP 487619, FR 2650955

END OF ANNEX